THE CHROMOSOMES OF *HYNOBIUS LEECHII* AND *H. NEBULOSUS* [1]

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(With 2 figures in text)

In previous papers (MAKINO, '32; INUKAI and MAKINO, '33) the author dealt with the chromosomes of hynobiid salamanders, making a comparative survey of five species: *Hynobius retardatus*, *H. lichenatus*, *H. tokyoensis*, *H. nigrescens* and *Salamandrella keyserlingii*. Recently the author has had the opportunity to study the chromosomes of two other species, *H. leechii* from Korea and *H. nebulosus* from Tottori and Nagasaki, the results obtained from which are given in the present paper [2].

It is a pleasure to the author to express here his hearty thanks to Professor OGUMA, under whose kind direction the work was carried out. Thanks are also due to Prof. T. INUKAI, through whose kindness the present material was supplied.

Hynobius leechii BOULENGER (Fig. 1)

The present species is the only representative of *Hynobius* living in Korea. The specimens employed in the study were collected in the vicinity of Keijo (Seoul), Korea, in May 1933, by Prof. K. SUZUKI of the Medical College, Keijo University, to whom the author wishes to express his sincere gratitude. They were brought to the laboratory alive and reared with scrupulous care for several months with the purpose of obtaining adequate material for the chromosome study.

The animals were killed at several different times during the interval from May to September. Those obtained in May proved to be favourable for the

1) Contribution No. 66 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.

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2) The general method for preparing sections was given in the author's previous paper ('32).

study of the spermatogonial chromosomes, while those in the latter half of July good for the spermatocyte chromosomes. As fixatives, BENDA's mixture and FLEMMING's strong solution without glacial acetic acid were applied, the former being chiefly employed for the preservation of the spermatogonial chromosomes.

As shown in Fig. 1, *a*, the spermatogonium contains fifty-six chromosomes. They arrange in a rosette form as usual in the equatorial plate; the larger ones take their position in the peripheral part of the spindle, surrounding those of smaller size in the central space.

The chromosomes can be classified into two main groups in respect to their shape. The one is a group of large V-shaped chromosomes and consists generally of ten homologous pairs varying in size and form. And they are confined to the periphery of the spindle directing their apices towards the center. The other group is composed of eighteen pairs of rod-shaped chromosomes of rather small size, most of which are scattered in the central space of the spindle, showing a gradatory difference of size. Thus the spermatogonial chromosome reveals a similar feature to that already reported in *H. tokyoensis* and *H. nigrescens* (MAKINO, '32), not only in number but also in morphological structure.

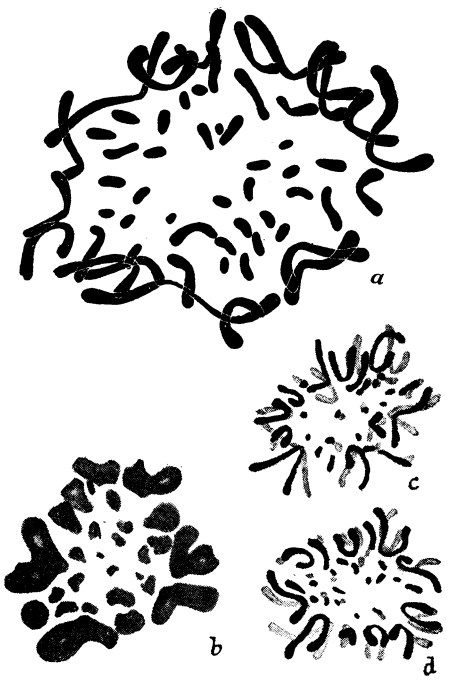

Fig. 1. *Hynobius leechii.* ×2500.
a, spermatogonial metaphase, 56 chromosomes.
b, primary spermatocyte metaphase, 23 tetrads.
c–d, secondary spermatocyte metaphase, 28 dyads; the paler chromosome denoting the identical monad with the deep black one.

As readily expected from the chromosome constitution of the spermatogonium, the metaphase of the primary spermatocyte shows, without any conflict, twenty-eight tetrads, of which ten or eleven larger ones usually take the peripheral position of the spindle, enclosing the remaining smaller ones at the central space (Fig. 1, *b*). They can hardly be divided into mega- and micro-tetrads as was done in *Megalobatrachus* (IRIKI, '32), since they present gradatory change in magnitude. Some larger tetrads disposed at the periphery take the form of a V, of which both arms represent a structure of ring-tetrad. They are probably derived from the large V-shaped chromosomes of the spermatogonium and seem

to be of a similar structure to those found in some other urodeles (*Diemyctylus* and *Megalobatrachus*, IRIKI, '32; SATO, '32) and some reptiles (NAKAMURA, '28; MATTHEY '31, '33).

In the equatorial plate of the secondary spermatocyte twenty-eight chromosomes are counted without exception (Fig. 1, *c-d*). As a whole, they consist of ten V-shaped and eighteen long and short rod-shaped dyads, showing a complete half set of spermatogonial chromosomes. They arrange in quite the same way as the latter. Every dyad appears as two identical monads superimposed horizontally on the equatorial plate.

Hynobius nebulosus (SCHLEGEL) (Fig. 2)[1]

Hynobius nebulosus is one of the common species and distributes widely through Honshu to Kyushu. The material for the study were secured in April, 1933 in Tottori (Southwestern Honshu)[2] and Nagasaki (Kyushu).[3] They were sent by mail to the laboratory alive and then the testes were fixed. For preservation of chromosomes FLEMMING's strong solution without glacial acetic acid was applied.

Fig. 2, *a* and *b*, shows the metaphase polar views of the spermatogonia, *a* being drawn from the material obtained from Nagasaki and *b* from Tottori. In both equatorial plates, there are contained fifty-six chromosomes, of which twenty represent V-shape and the remaining thirty-six are rod-shaped, arranged in a fairly rosette form. Most of the chromosomes exhibit longitudinal splits for provision of the ensuing division.

In the number, arrangement and general morphological characters of individual chromosomes, on the whole, one can hardly find any remarkable difference between the present species and *H. tokyoensis*, *H. nigrescens* and *H. leechii*.

Fig. 2. *Hynobius nebulosus*. ×2500. *a*, *b*, spermatogonial metaphase, 56 chromosomes.

1) Identification was done by Mr. I. SATO of Hiroshima University, to whom the author wishes to express his hearty thanks.

2) 3), For collection of the specimens the author is greatly indebted to Messrs. K. KAWADA and D. NAKAMURA.

Literature

INUKAI, T. and MAKINO, S. 1933. On a local form of *Hynobius lichenatus* BOUL. inhabiting Akita and Yamagata Prefectures *(Japanese)*. Rep. Jap. Assoc. Adv. Sci., Vol. 8.

IRIKI, SH. 1932. Studies on amphibian chromosomes. 6. On the chromosomes of *Diemyctylus pyrrhogaster*. Sci. Rep., Tokyo Bunrika Daigaku, Sec. B. Vol. 1.

———— 1932. Studies on amphibian chromosomes. 7. On the chromosomes of *Megalobatrachus japonicus*. Ibid. Vol. 1.

MAKINO, S. 1932. The chromosome number in some salamanders from northern Japan. Journ. Fac. Sci., Hokkaido Imp. Univ., Ser. VI. Vol. 2.

MATTHEY, R. 1931. Chromosomes de reptiles sauriens, ophidiens, cheloniens. L'évolution de la formule chromosomiale chez les sauriens. Rev. Suisse de Zool., T. 38.

———— 1933. Nouvelle contribution à l'étude des chromosomes chez les sauriens. Rev. Suisse de Zool., T. 40.

NAKAMURA, K. 1928. On the chromosomes of a snake, *Natrix tigrina*. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B. Vol. 4.

SATO, I. 1932. Chromosome behavior in the spermatogenesis of urodele amphibia, *Diemyctylus pyrrhogaster* (BOIE). Journ. Sci., Hiroshima Univ., Ser. B. Div. 1. Vol. 2. Art. 3.

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